08-SCA-YALE-005

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Functional use of Embryonic Stem Cells for Kidney Repair**

Amount requested: $200,000; Amount funded: $200,000

Principal Investigator: **Lloyd G. Cantley**
Institution: **Yale University**

Description: **This Project's purpose is to define the conditions necessary to generate renal progenitor cells from embryonic stem cells and to use those cells to repair injured kidneys.**

Project Summary: The kidney functions to clear the blood of toxins and to maintain internal fluid and electrolyte homeostasis. These properties result in a marked sensitivity to reductions in blood flow, with resultant tubular cell necrosis and acute renal failure. Repair of this injury is dependent on surviving tubular cells. However, in older or severely ill patients this repair process is often insufficient, leading to chronic kidney failure, dialysis and frequently death. The current proposal is designed to develop a strategy for priming of embryonic stem (ES) cells to become kidney progenitor cells that could be used in the treatment of patients with acute renal failure in whom endogenous tubule repair is either delayed or absent. While this work will transition to human ES cells in its later stages, the initial development of strategies to prime ES cells to adopt a renal epithelial cell fate, and the testing of methods of delivery of these cells to the injured kidney will be performed using mouse ES cells and mouse models of acute and chronic kidney injury currently performed in the laboratory. In the first specific aim, several approaches will be tested for the priming of ES cells towards adopting a kidney specific fate. These will include culture of ES cells in a sequential cocktail of growth factors and cytokines and co-culture of ES cells with explanted embryonic kidney. In the second aim, primed ES cells will be tested for their ability to home to and functionally incorporate into damaged kidneys by comparing intravenous injection, intra-arterial injection, intra-ureteral injection and direct injection into the kidney in mouse models of kidney injury. Thus these studies address a novel approach to the treatment of kidney disease by providing pathways to enhance kidney repair even after the acute injury has occurred.

O8-SCA-UCHC-009

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Cytokine-induced production of transplantable hematopoietic stem cells from human ES cells**

Amount requested: $200,000; Amount funded: $200,000

Principal Investigator: **Laijun Lai**
Institution: **University of Connecticut Health Center**
Collaborator: Irving Goldschneider

Description: **This Project's purpose is to induce human embryonic stem cells to produce transplantable hematopoietic stem cells *in vitro* and to demonstrate the ability of the latter cells to establish long-term hematopoietic reconstitution *in vivo* in recipient mice.**

Project Summary: Hematopoietic stem cell transplantation (HSCT), the most common cell-based therapy applied today, is widely used in the treatment of cancer, aplastic anemia, complications of irradiation and chemotherapy, primary (hereditary) and secondary (acquired) immunodeficiency disorders, organ transplantation and autoimmunity. Bone marrow, umbilical cord blood, and mobilized peripheral blood are the major sources of hematopoietic stem cells (HSCs). However, especially in adult patients, HSCT is frequently limited by the unavailability of sufficient freshly harvested HSCs and by the inability to reliably expand the number of transplantable HSCs from these sources *in vitro*. Therefore, the evaluation of alternative sources of cells for HSCT remains an important goal that will be pursued in the present proposal by using human ES cells.

08-SCA-YALE-010

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Vrk1-mediated regulation of p53 in the human ES cell cycle**

Amount requested: $200,000; Amount funded: $200,000

Principal Investigator: **Valerie Reinke**
Institution: **Yale University**

Description: **This Project's purpose is to dissect the mechanisms that regulate the activity of a key tumor suppressor protein, p53, in human ES cells, so that ultimately, the quality and function of ES cells will be optimized for therapeutic purposes**

Project Summary: The use of ES cells to investigate disease and develop therapies requires genetic manipulation of the ES cells in culture. Extensive culture of undifferentiated ES cells can result in genomic instability, decreasing pluripotentiality and functionality. Ultimately, a major concern of using ES cells in therapies is the possibility that damaged or undifferentiated ES cells might form tumors if inadvertently introduced into the body. Controlling the activity of the tumor suppressor protein p53 may provide a way to avoid these complications. p53 acts in many cell types to induce cell death or cell cycle arrest upon DNA damage, but p53 does not respond to DNA damage in undifferentiated ES cells, despite being present at high levels. Only when ES cells are induced to differentiate do they display a p53-dependent cell cycle arrest checkpoint. However, known inhibitors of p53 in other cell types do not act in ES cells. Therefore, some novel mechanism must exist in undifferentiated ES cells to hold p53 activity in abeyance. We hypothesize that the kinase Vrk1 inhibits p53 in ES cells, based on the following facts: (1) human Vrk1 is expressed in ES cells, (2) human Vrk1 can phosphorylate p53 in vitro, (3) loss of Vrk1 causes a cell cycle arrest in both mouse embryonic blastomeres and in *C. elegans* germline stem cells, and 4) loss of p53 suppresses the Vrk1 mutant phenotype in *C. elegans* stem cells. We propose two aims to investigate the roles of p53 and Vrk1 in regulating human ES (hES) cell proliferation and genomic stability. The first aim will characterize the role of p53 in human ES cells. We will monitor p53 levels and activity during the cell cycle and in response to DNA damaging agents. The second aim investigates VRK1 as a negative regulator of p53 in hES cells. We will use RNAi to decrease Vrk1 and p53 levels in hES cells to test whether the absence of VRK1 results in increased activity of p53 and p53- dependent alterations in cell cycle kinetics, apoptosis, and genome stability. These experiments will dissect the requirements for p53 in human ES cells and establish conditions under which these cells can maintain their critical pluripotent properties without accumulating harmful mutations.

08-SCA-YSME-011

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Cortical neuronal protection in spinal cord injury following transplantation of dissociated neurospheres derived from human embryonic stem cells**

Amount requested: $200, 000; Amount funded: $200,000

Principal Investigator: **Masanori Sasaki, MD, PhD**
Institution: **Yale University School of Medicine**

Description: **This Project's purpose is to study the potential neuroprotective effect of stem cell transplantation into the injured rat spinal cord.**

Project Summary: Spinal cord injury (SCI) results in dysfunction due to disruption of motor signals from brain to spinal cord. We recently demonstrated that transplantation of gene-modified human mesenchymal stem cells to secrete the neurotrophic factor BDNF (BDNF-hMSC) from human bone marrow could inhibit apoptosis in the motor cortex after experimental SCI in rats, which could contribute to repair and functional recovery. Neural progenitor cells dissociated from neurosphere have an ability to differentiate into neurons and glia in vivo and vivo. Importantly, neurospheres have been prepared from human embryonic stem cells (hESCs). Although several mechanisms have been suggested including neurogenesis, regeneration, axonal sprouting, recruitment of endogenous Schwann cells and remyelination, there is considerable evidence suggesting that, under appropriate cell preparation and transplantation conditions, functional outcome in experimental SCI can be enhanced by cellular transplantation. Questions still remain with regard to cellular mechanisms responsible for improvement in functional outcome. This project will be to evaluate a potential additional role of neuroprotection by the supraspinal effects of transplantation of spinally transplanted neurosphere derived from hESCs to promote functional recovery after spinal cord injury (SCI). We will determine if transplantation of dissociated neurospheres derived from hESCs (hNSs) results in improved functional outcome and anti-apoptosis effects in M1 cortex in the brain. We will carry out three Specific Aims (SA): SA1: Functional recovery following transplantation of hNSs after SCI in rats. SA2: Evaluation of remote supraspinal effects of hNSs on reducing apoptotic cell death and increase cell survival of M1 cortex following SCI. SA3: Structural evaluation of the spinal cord after transplantation of hNSs into SCI. Success of this project using hMSCs would provide important preclinical work for the consideration of human clinical studies for SCI.

08-SCA-YALE-019

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Molecular Control of Pluripotency in Human Embryonic Stem Cells**

Amount requested: $200,000; Amount funded: $200,000

Principal Investigator: **Natalia Ivanova, PhD**—Assistant Professor, Yale Stem Cell Center and the Department of Genetics
Institution: **Yale University**
Collaborators: Caihong Qiu, PhD—Technical Director of the hESC Core, Yale Stem Cell Center; Daniel DiMaio, PhD— Professor, Vice Chairman of the Department of Genetics

Description: **This Project's purpose is to identify key genes that control pluripotency in human embryonic stem cells**

Project Summary: Embryonic stem (ES) cells promise to revolutionize medicine. If we determine how to control the expansion and differentiation of human ES cells, then we could produce cells of any human organ at will. In order to achieve this goal we need to gain a deep understanding of how cell fate decisions such as self-renewal, differentiation and cell death are controlled in these cells. Studies in the mouse have provided insights into the molecular regulation of ES cells. However, the biological equivalence of mouse and human ES cells remains unclear. While some regulatory components are functionally conserved, others appear to be species-specific. There are differences in morphology, patterns of embryonic antigen expression, cytokine dependence and cell cycle kinetics. These findings suggest that data accumulated in the mouse system cannot be extrapolated directly to human ES cells. It is likely that different constellations of genes are involved in the regulation of human ES cells. In this grant, I propose to identify, in a comprehensive manner, molecular components and pathways that control pluripotency in human ES cells using a direct shRNA-based functional screen. This approach was developed during my postdoctoral training and has been successfully applied to study mouse ES cells. These studies will provide data and reagents that should allow further analyses of human ES cells at multiple molecular and biochemical levels. In addition to fundamental insights into cell fate control, these studies will extend our ability to develop therapeutic strategies for the treatment of various human diseases.

08-SCA-YALE-022

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Regulation hESC-derived neural stem cells by Notch signaling**

Amount requested: $188,676, Amount funded: $188,676

Principal Investigator **Joshua Breunig, Ph.D.** (P.I.),
Pasko Rakic, M.D., Ph.D. (Sponsor)
Institution: **Yale University School of Medicine**

Description: **This Project's purpose is to determine the role of Notch signaling in the cell fate (glial/stem cell vs. neurons) and maturation (arborization) of neural stem cells derived from human embryonic stem cells.**

Project Summary: The mammalian central nervous system (CNS) has a relatively limited capacity for self repair when compared with other vertebrates. Except for two discrete regions, neurons are not replaced after neurotrauma or disease. Embryonic stem cells have the capacity to generate all of the cell types of the CNS, including a diverse array of neuronal subtypes. The molecular mechanisms governing these cell fate choices are poorly understood. We and others have found that a receptor protein, Notch, plays a critical role in the determination of glial vs. neuronal fate of the stem cell progeny in the postnatal CNS. Furthermore, it seems to be actively involved in the blockade of neurogenesis in the adult brain, limiting regeneration. In addition, in another context, Notch often works in concert with other molecules that are able to initiate the expression of specific genes, and thus are able to regulate more specific aspects of cell fate, such as the extent of neuronal arborization. The intent of this project is to overexpress Notch and block its function in parallel experiments to determine the role of Notch both at the neural stem cell (NSC) level and at the maturing neuron stage. If the role of Notch is conserved in human NSCs, increasing activated Notch levels should increase the numbers of NSCs while blocking Notch signaling is expected to yield an enriched population of postmitotic cell types, including neurons. These differentially treated cells will be transplanted into mice in order to determine the effect of the in vivo environment on cell fate choices as well as the potential clinical feasibility of this approach for repairing neural tissue. In alternate experiments, similar gain- and loss-of-function experiments will be used to examine the role of Notch in neuronal arborization. Proteomic and gene expression screens will be used to determine the molecular targets of Notch signaling in both contexts.

08-SCA-YALE-031

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Definitive Hematopoietic Differentiation of Human Embryonic Stem Cells Under Feeder-Free and Serum-Free Conditions**

Amount requested: $200,000; Amount funded: $200,000

Principal Investigator: **Qiu Caihong, Ph.D.**
Institution: **Yale University**

Description: **The goal of this project is to develop an efficient and animal product-free hESC differentiation protocol to understand the mechanisms of early human development and clinical application.**

Project Summary: Human embryonic stem cells (hESCs) represent an excellent tool for scientists to learn about how we develop in the womb. These cells are also very useful for applications in tissue engineering and drug screening. Much research is focused on differentiating hESCs into pure populations of different cell type. We propose to develop approaches to efficiently induce hESCs into blood cells in a system that is free of any non-human products. This has never been done before, but it is important to remove animal serum and animal cells from the hESC growth conditions so that the cells can be used for humans in the future. Specifically, we would like to be able to produce bone marrow cells and red blood cells that could be used for transplantations and transfusions respectively. In addition to the important benefits to patients, our studies will also help us to better understand how blood cells form. There are many stem cell researchers throughout Connecticut who would like to be able to induce hESCs to form blood and related cell types, and we will gladly share our findings with them in order to facilitate their research.

O8-SCA-UCHC-033

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project**: Differentiation of human ES cell lines to neural crest derived trabecular meshwork like cells – implications in glaucoma**

Amount requested**:** $200,000; Amount funded: $200,000

Principal Investigator**: Dharamainder Choudhary, Ph.D.**
Institution: **University of Connecticut Health Center**
Collaborators: Ren-He Xu, M.D. Ph.D., John B. Schenkman, Ph.D.

Description: **This purpose is to develop methods to generate trabecular meshwork (TM) like cells to replace defective ones that play a critical role in development of glaucoma disease.**

Project Summary: Glaucoma is the major cause of blindness worldwide. One of the major risk factors for development of glaucoma is an elevated intraocular pressure (IOP). This develops due to resistance to the aqueous humor outflow in the TM region of eye. The treatment generally constitutes of either to decrease the synthesis rate of aqueous humor or increase the outflow by performing surgery to cannulate the pathway. These treatments are not permanent and patients require repetitive surgeries in many cases. Human embryonic stem (ES) cells offer a unique advantage of generating a differentiated cell line of TM cells which can be targeted for transplantation in the anterior chamber, to replace the damaged TM cells and populate the structure with the healthy TM cells. Although this is a distant goal it can be accomplished, and the first aim of the current proposal is to develop the optimal conditions for differentiating human ES cells to a cell type which displays characteristics similar to TM cells. This will involve the coculture of ES cells with a stromal cell line for induction of differentiation and isolating the mesenchymal precursor cells using CD73-labelling. The second aim is focused more towards elucidating the role of the CYP1B1 gene and other glaucoma causing genes in the process of TM cellular development during ontogeny. We found mutations in the CYP1B1 gene in Primary congenital glaucoma (PCG) patients. PCG is most common form of glaucoma during infant stages and characterized by bulging eyes due to high IOP. CYP1B1 is highly conserved in vertebrate species and Cyp1b1(-/-) null mice display similar abnormalities in the TM as seen in human PCG eye, suggestive of critical role of CYP1B1 in early eye development. We have recently reported the expression pattern of CYP1B1 protein in the eye at various stages of fetal and postnatal development stages and found the expression at discrete locations at specific times of development. The aim will involve studying the changes in expression levels of the glaucoma causative genes at three stages, undifferentiated, mesenchymal precursors and differentiated TM-like cells. Some recent reports have shown the use of the embryonic stem cells for regeneration of retinal cells and its potential application towards reducing the vision loss. The TM is the primary key lesion area which is damaged in most cases of glaucoma and responsible for resistance to aqueous humor outflow resulting in elevated IOP and stress on the ocular cells. Our novel approach of targeting the TM cells derived from mesenchymal cells of neural crest origin, has a strong potential to act as a viable future glaucoma treatment strategy.

08-SCA-YALE-036

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **The role of the piRNA pathway in epigenetic regulation of human embryonic stem cells**

Amount requested: $200,000.00; Amount funded: $200,000

Principal Investigator: **Qiaoqiao Wang, Ph.D**, (Haifan lin, Ph.D)
Institution: **Yale University**

Description: **This project investigates how a new class of small RNAs called piRNAs control the self-renewal and pluripotency of human embryonic stem cells.**

Project Summary: Human embryonic stem cells hold great potential in regenerative medicine because they are the only type of cells that have the ability to self-replicate and to generate all types of body cells (*i.e.,* pluripotency). To harness this potential, we first need to understand how the self replication and pluripotency of these stem cells are controlled by their genes. Here, we propose to study this question by investigating how a new class of small RNAs called piRNAs control the self-replication and pluripotency of human embryonic stem cells. piRNAs were recently discovered by our lab and others. Our latest studies on piRNAs in stem cells of model organisms show that piRNAs play key role in switching on and off the activity of different genes in the cell. Such a key role has not been discovered or even been suggested before. More specifically, we found out that piRNAs switch on and off different genes by forming complexes with a class of proteins called Piwi proteins. Different piRNA-Piwi complexes directly bind to their corresponding target genes to turn them on or off. Because the basic ways of gene activity switching is the same in all examined organisms, we think that these piRNA-Piwi switches will also play a key role in controlling gene activity in human embryonic stem cells, which in turn define these cells' ability to self-replicate and to produce different types of body cells. To test this hypothesis, we propose to isolate piRNAs from human embryonic stem cells. Once we have these piRNAs and determine their genetic information (*i.e.,* sequence), we will immediately know what genes in these stem cells are likely controlled by these piRNAs (Aim 1). We will then test whether these piRNAs indeed control human embryonic stem cells by inactivate their partner, Piwi proteins, in these cells. Because piRNAs are not functional without Piwi proteins, this inactivation will effectively eliminate piRNA function in stem cells. Now, if the stem cells can no longer self-replicate and/or produce all types of body cells as we expected, we will know definitively that piRNAs are important for human embryonic stem cells (Aim 2). We will then further study what genes in stem cells are bound and controlled by the piRNA-Piwi complexes (Aim 3), which will allow us to use specific piRNAs to switch on and off different genes to control the stem cell behavior for medical applications in the future.

O8-SCA-UCON-040

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Early differentiation markers in human ES cells: identification and characterization of candidates**

Amount requested: $200,000; Amount funded: $200,000

Principal Investigator **Mark G. Carter, Ph.D.**
Institution: **Center for Regenerative Biology, University of Connecticut**
Collaborator: Tomokazu Amano, Ph.D. – Research Associate, Center for Regenerative Biology

Description: **This Project's purpose is to identify genes which may control early differentiation events in human ES cells, and to characterize their regulation and function.**

Project Summary: The presence of heterogeneous cell populations in mouse embryonic stem cell (mESC) cultures is becoming increasingly recognized, as recent reports have demonstrated that cell surface marker genes, such as *Ssea1*, *Pecam1*, and *Icam1*, are expressed heterogeneously under non-differentiating culture conditions, and their expression patterns appear to be connected to differentiation state and developmental potential. While such cell surface antigens are convenient markers of differentiation state, their expression is the end result of upstream differentiation processes controlled by transcription factors. Screening for heterogeneously-expressed transcription factors could identify genes controlling early differentiation events, both in ES cell (ESC) systems and early embryonic development.
Recently, we reported that *Zscan4*, a transcription factor which is expressed exclusively at the two-cell stage in mouse embryos, is also heterogeneously expressed in undifferentiated mESC cultures, with ≤ 10% of cells positive for expression by *in situ* hybridization. Subsequently, we identified a panel of over 300 candidate genes (predominantly known or putative transcription factors), and an *in situ* hybridization screen of these genes in undifferentiated mES cultures identified several genes with heterogeneous expression in a minority of cells. Functional characterization of these genes in the mouse is underway, in an effort to determine what roles these genes may play in the promotion or suppression of cellular differentiation in pluripotent cells. We propose to use a similar approach to screen mESC candidates for heterogeneous expression in human ES cells (hESC), identify transcription factor genes which are heterogeneously-expressed only in hESC cultures, and develop live-cell reporter systems to allow purification and characterization of expressing and non-expressing cells from heterogeneous cultures for further characterization. By extending this approach to hESC, we hope to identify markers for / effectors of cellular differentiation which will be more relevant to the challenges of efficiently directing *in vitro* differentiation of hES cells towards therapeutically useful, transplantable derivatives.

O8-SCB-UCON-006

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Synaptic Replenishment Through Embryonic Stem Cell-Derived Neurons in a Transgenic Mouse Model of Alzheimer's Disease**

Amount requested: $499,813; Amount funded $499,813

Principal Investigator: **Ben A. Bahr, Ph.D.**
Institution: **University of Connecticut**

Description: **This Project's purpose is to discover and develop ways to improve the viability of transplanted hESC-derived neurons in the aged brain, in order to offset the cognitive decline of age-related disorders.**

Project Summary: While there is great interest in the application of stem cells, little is known about their viability and related cell death mechanisms in the context of transplantation in the aged brain. One of our challenges is to address the relatively poor survival of implanted embryonic neurons, as shown by earlier studies indicating significant cell death within a few days of intracerebral implantation. Necrotic and other pathogenic processes acting on transplants in young adults have been identified in recent years, and we hypothesize that by blocking those pathways that are most pronounced in the aged brain, it will become possible to enhance survival of transplanted hESC-derived neurons and their effectiveness to offset age3-related synaptopathogenesis. This proposal then focuses on a set of experiments to test stable transplants for improving synaptic plasticity marker levels and behavioral performance in aged APPswe/PS1dE9 transgenic mice, an established model of Alzheimer's disease. We will also work to develop hESC-derived neurons for screening agents that enhance transplant stability and synaptic maintenance signals, or novel lines resistant to cell death pathways.

The goals of this proposal are to test the hypothesis that stable transplantation of neurons derived from embryonic stem cells causes local attenuation of Alzheimer-type synaptic decline, and to develop a human cell model for screening compounds that enhance transplant stability and thus therapeutic potential.

The three objectives of the project are:

1. To determine the viability of neurons derived from embryonic stem cells after being transplanted into the cerebral cortex and hippocampus of aged mice, as well as the pathogenic processes that influence their viability.
2. To test conditions that enhance the viability of transplanted neurons derived from embryonic stem cells for effectiveness in reducing synaptic decline in the APPswe/PS1dE9 transgenic mouse model of Alzheimer's disease.
3. To develop hESC-derived neurons for the screening of novel agents which enhance transplant stability and synaptic maintenance signaling for the treatment of dementias.

Together these experiments should lead to important insights into how to slow the synaptic decline and associated cognitive deficits of human dementias including AD.

O8-SCB-UCHC-011

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Human embryonic stem cells (hESC) as a source of radial glia, neurons and oligodendrocytes**

Amount requested: $500,000; Amount funded: $500,000

Principal Investigator: **Nada Zecevic, MD, PhD**
Institution: University of Connecticut Health Center
Collaborators: Xue-Jun Li, PhD, and Srdjan Antic, MD

Description: **This Project's purpose is to generate radial glia cells from hESC lines (H9) to characterize them and study the effect of Pax6 and Olig2 in their differentiation into neurons and oligodendrocytes, respectively.**

Project Summary: Understanding the molecular and physiological determinants of cortical neural progenitor cells is essential for understanding the development of the human brain in health and in disease. We previously established methods of isolating and differentiating radial glia cells (RG) from human fetal brains (fetal RG) and characterized these cells in respect to transcription factors that influence their development (Mo et al., 2007, Mo and Zecevic, 2007). Fetal progenitor cells, however, are difficult to culture and propagate, and numerous ethical and practical problems exist in harvesting these cells. Thus, hESC lines have several advantages: a) to study developmental processes of neurogenesis and specification of cell subtypes; b) drug screening and disease modeling; and finally, c) to generate a larger number of progenitors necessary for future cell therapies. Careful characterization of these cells is necessary prerequisite to their use in therapies, to avoid risk of tumor formation. Thus, the exact stage of development for grafting has to be carefully determined.
We are proposing to study whether hESCs could be used as a source of human RG cells that can be differentiated into either neurons or oligodendrocytes. We will compare whether hES-RG cells have the same cellular characteristics (molecular, morphological, electrophysiological) as well as proliferation and differentiation potentials as the fetal RG. This knowledge is necessary for better understanding the development of the human brain and for creating novel cell therapies for neurodegenerative processes.

O8-SCB-UCHC-012

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Tyrosine phosphorylation profiles associated with self-renewal and differentiation of human embryonic stem cells**

Amount requested: $500,000; Amount funded $450,000

Principal Investigator: **Bruce J. Mayer, Ph.D.**
Institution: **University of Connecticut Health Center**
Collaborators: Consultants: Peter Nollau, Universitaetsklinikum Hamburg-Eppendorf (Hamburg, Germany); Brenton Graveley (UCHC); Ren-He Xu (UCHC); David Han (UCHC)

Description: **This Project's purpose is to identify changes in the phosphorylation of cell proteins that are likely to play a role in human embryonic stem cell self-renewal, survival, and differentiation.**

Project Summary: A fundamental property of human embryonic stem cells is their ability to survive and renew themselves indefinitely in the laboratory, and then under appropriate conditions to differentiate into cells that can perform essential functions in the body, such as neurons or muscle cells. It is this ability to self-renew on the one hand, and then differentiate into many different adult cell types on the other, that makes human embryonic stem cells an extremely promising avenue for treating currently incurable human diseases and health problems (regenerative medicine). The aim of this project is to understand the mechanistic details of how this switch between self-renewal and differentiation is controlled in the cell. This knowledge will allow us to manipulate human embryonic cells in a more rational way to generate specialized cells that can be used to treat patients. It is known that one of the mechanisms controlling this process is tyrosine phosphorylation, which is the addition of a phosphate group to the amino acid tyrosine in proteins. Unfortunately, despite their importance, tyrosine phosphorylated proteins are present in the cell at vanishingly low levels, making it very difficult to study and characterize them. The investigator's group has recently developed a new and highly sensitive method to profile the entire spectrum of tyrosine phosphorylated proteins in a cell sample, termed SH2 profiling. This new method will be used to profile the phosphorylation patterns in human embryonic stem cells under conditions that affect self-renewal, survival, and differentiation. Based on these results, the investigators will go on to identify specific phosphorylated proteins that play a key role in these cell fate decisions. By applying a new, cutting-edge proteomic method to human embryonic stem cells, important new insights into how to manipulate human stem cells for therapeutic purposes will be gained.

O8-SCB-YALE-013

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Effect of hypoxia on neural stem cells and their function in CNS repair**

Amount requested: $499,746; Amount funded: $449,771.40

Principal Investigator: **Vaccarino, Flora M.**
Institution: **Yale University**
Collaborators: Laura Ment, M.D; Anna Szekely, M.D., Ph.D.; Heping Zhang, Ph.D.

Description: **This Project's purpose is to analyze changes in gene expression in neural stem cells after injury and their relevance in promoting recovery.**

Project Summary: Neural stem cells (NSCs) can repair the brain after injury, but this repair, when present, is invariably incomplete. In this proposal we plan to identify the changes in gene expression that enable NSCs to repair the injured brain and understand their role for human NSC development. We have developed a mouse model of neonatal hypoxia which causes brain injury manifested by a loss of cortical neurons. Consistent with the observation that the outcome of neonatal injury is generally better than adult injury, this loss of cortical neurons is subsequently repaired. We have established that repair occurs through the proliferation of astroglial NSCs (expressing Glial Fibrillary Acidic Protein, or GFAP) which then differentiate into new cortical neurons, astrocytes and oligodendrocytes 3-4 weeks after the insult. In this proposal, we will target the enhanced green fluorescent protein (EGFP) gene to GFAP+ cells via inducible Cre recombination in vivo. The permanent EGFP expression in GFAP+ cells allows us to track their progeny following hypoxia. EGFP+ cells will be isolated after hypoxic insult to analyze their gene expression profile. By contrasting and comparing genes changes in GFAP+ cells isolated from neurogenic versus non-neurogenic regions, we will select genes whose changes closely predict the therapeutic potential of the GFAP+ NSCs. These changes will be validated by examining the expression of the candidate gene/proteins in GFAP+ cells within the neurogenic areas *in vivo* following hypoxia. The validated genes will be overexpressed or knocked-down in human NSCs (generated from both federally approved and non-approved human ES cell lines) using lentiviral shRNA. The induced changes in the human NSC phenotype will be characterized via a high-throughput cell-based imaging system. A selected number of genes that elicit changes in expression of differentiation markers in human NSCs will be identified. This project will elucidate key molecular mechanisms underlying the capability of NSCs to generate neuronal progenitors and differentiated neurons, and consequently foster recovery from brain injury. The study will allow the future development of genetically modified human NSC lines and mouse in vivo mouse models that will further consolidate the role of these NSC genes in fostering brain recovery after injury.

O8-SCB-UCHC-016

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Directed differentiation of embryonic stem cells into cochlear precursors for transplantation as a treatment of deafness.**

Amount requested: $ 500,000; Amount funded: $450,000

Principal Investigator: **D. Kent Morest, MD**
Institution: University of Connecticut Health Center

Description**: This Project's purpose is to grow stem cells from human embryos in a tissue culture dish and expose them to chemicals which will turn them into functional nerve cells that can be transplanted into a previously deaf cochlea, connect with the brain and restore hearing.**

Project Summary: Deafness and hearing loss caused by damage to the inner ear from noise, aging, drugs, or infections are major, and as yet incurable diseases. Hearing loss and ringing in the ears often follow a progressive course to deterioration in the quality of life and personal isolation. Our long-term goal is for a therapy that replaces the degenerated sensory cells (hair cells) and nerve cells (ganglion) of the inner ear and auditory part of the brain (cochlear nucleus) with newly formed cells derived from human embryonic stem cells (hESC). We have found a way to promote maturation of embryonic stem cells from mice (mESC) and direct them to become precursor cells of the cochlear ganglion. To date we have generated mESC in which a fluorescent marker is inserted into a specific gene (called Gbx2). As they develop, the precursor cells become visible due to the fluorescent marker, showing that these cells specifically express the Gbx2 gene. This allows us to sort out the cells destined to become precursor cells and to culture them alongside normally developing cochlear tissue under a maturation protocol. This protocol uses a growth hormone (called FGF2) that normally occurs in human and mouse embryo brains and ears. We stick FGF2 molecules to tiny latex beads, which are inserted into the cultured precursor cells. We have succeeded in transplanting such cultured precursor cells into the infant and adult mouse cochlear nucleus, and the infant cochlea, where they developed as normal-appearing nerve cells in the course of two weeks. Transplanting these cells to the inner ear of postnatal mice, we have shown that they survive well past the age for the onset of hearing and send new nerve fibers to hair cells in the damaged cochlea and into the cochlear nucleus. We will show the functioning of transplants with hearing tests in mice previously deafened by noise, chemicals, or mechanical ablation. If this is successful, we will repeat the process with hESC. This research provides models and strategy for future clinical trials to cure deafness by transplantation of hESC.

O8-SCB-UCHC-021

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Targeting Lineage Committed Stem Cells to Damaged Intestinal Mucosa**

Amount requested: $500,000; Amount funded: $450,000

Principal Investigators: **Daniel W. Rosenberg** and Charles Giardina
Institution: University of Connecticut Health Center
Collaborator: Alex Lichtler

Description: **Our long-term goal is to develop stem cell technology for the repair of tissue damage associated with longstanding ulcerative colitis (UC), irradiation and other injuries.**

Project Summary: The intestinal mucosa, comprised of cells from all three embryonic lineages, provides an excellent experimental system for studying tissue renewal and repair [1]. Our work will require the development of new methodologies for working with human embryonic stem cells (hESCs) that induce their lineage commitment into multi-potent intestinal stem cells. We hypothesize that lineage committed stem cells will migrate and home to the damaged intestinal epithelium, undergo engraftment, differentiation and finally reconstitution into a fully functional colonic mucosa displaying all four colonocyte lineages. Our work will be facilitated by the newly identified intestinal stem cell marker, Lgr5, which was reported in October by Hans Clevers and colleagues [2]. This cell surface marker will enable us for the first time to identify early committed intestinal stem cells and test them for their therapeutic potential.

O8-SCB-UCHC-022

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Modeling Motor Neuron Degeneration in Spinal Muscular Atrophy Using Human Embryonic Stem Cells**

Amount requested: $500,000; Amount funded: $450,000

Principal Investigator: **XUEJUN LI**
Institution: **University of Connecticut Health Center**
Collaborator: ZHAOWEN WANG

Description: **This project's purpose is to use human embryonic stem cells to model the pathological processes that occur in spinal muscle atrophy caused by the reduced level of survival motor neuron protein so that treatments can be developed.**

Project Summary: Spinal muscular atrophy, one of the most common autosomal recessive diseases, is caused by the reduced level of survival motor neuron (SMN) protein that results from loss or mutation of the SMN1 gene. The hallmark of this disease is the degeneration of spinal motor neurons and subsequently muscular atrophy, leading to the death of more than half of those afflicted by age two. There is no effective treatment for this disorder, primarily due to the lack of an experimental system for understanding why human motor neurons are specifically susceptible to diminished levels of SMN protein and for screening effective therapeutic agents. This application, built upon our successful generation of spinal motor neurons from human embryonic stem cells (hESCs), aims to model the motor neuron degeneration that occurs in spinal muscular atrophy through modifying hESCs. First, we intend to establish stable hESC lines with a deficiency in SMN protein levels by reducing levels of SMN expression using RNA interference. Spinal motor neurons will be differentiated from these hESC lines and a control line and assayed for a variety of functional changes including survival, neurite outgrowth, ability to form synaptic connections with muscle cells, apoptosis and cell death. Notably, by comparing the responses of motor neurons and other neurons to reduced levels of SMN protein, we will be able to understand why motor neurons are affected in this disorder. The successful establishment of such a human cell model has the potential to greatly advance research and treatment of spinal muscular atrophy. Significantly, our system will provide a unique platform with which high-throughput drug screening may pinpoint compounds to treat this debilitating and fatal genetic disorder.

O8-SCB-YSME-026

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Wnt signaling and cardiomyocyte differentiation from human embryonic stem cells**

Amount requested: $496,465; Amount funded: 446,818.50

Principal Investigator: **Dianqing Wu**
Institution: Yale University School of Medicine

Description: **This Project's purpose is to investigate Wnt signaling in cariomyocyte differentiation of hESCs and engraftment.**

Project Summary: Studies have strongly implicated Wnt signaling in cardiogenesis. Although the precise involvement of Wnt signaling in each step of the differentiation from embryonic stem cells (ESCs) to cardiomyocytes is not clear, studies from a number of organisms suggest that canonical Wnt signaling promotes the differentiation to the mesoderm, while noncanonical or inhibition of canonical Wnt signaling promotes cardiomyocyte specification and differentiation. Preliminary studies of hESCs in my lab as well as other labs suggest that Wnt regulation of cardiomyocyte differentiation may be highly conserved between mouse and human ESCs. We hypothesize that differential manipulation of Wnt signaling at different stages of hESC-cardiomyocyte differentiation can be exploited to enhance production of cardiogenic cells from hESCs and that cardiogenic precursor cells may be more suitable for cardiac implantation and repair in vivo. In the first specific aim, we plan to systemically dissect the involvement of Wnt signaling in hESC-to-cardiomyocyte differentiation in culture by investigating the role of Wnt signaling in hESC differentiation to the mesoderm, to cardiogenic precursor cells, and to mature cardiomyocytes. Cells will be treated with reagents that stimulate and inhibit canonical and non-canoncial Wnt signaling, and specific marker gene expression will be examined by QRT-PCR. Functional and structural phenotypes of terminally differentiated cells will also be characterized. The second specific aim is to test if cardiogenic progenitor and cadiomyomyocyte precursor cells, when transplanted, improve cardiac function recovery in a myocardiac infarction (MI) mouse model. A novel biodegradable synthetic scaffold, which has been successfully used in generating artificial blood vessels, will also be tested for facilitating the engraftment. The fate of transplanted cells will be characterized by general pathology and immunohistochemistry. Cardiac functional improvement will be evaluated by electrocardiography, measurements of cardiac pressure, and echocardiography. By taking advantage of our extensive expertise in Wnt signaling, the possession of unique small molecule compounds that regulate Wnt activity, and a team of cross-discipline co-investigators who have expertise in biomaterial sciences and cardiac surgery, we will better understand the role of Wnt signaling in hESC-to-cardiomyocyte differentiation and establish a method for efficienct cardiogenic cell production and engraftment, which lead to cardiac function recovery in a heart disease model.

O8-SCB-YSME-025

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: Human embryonic and adult stem cells for vascular regeneration

Amount requested: $500,000; Amount funded: $450,000

Principal Investigator: **Laura E. Niklason, MD/PhD**
Institution: **Yale University School of Medicine**
Collaborators: Caihong Qiu, PhD; Zhaodi Gong, MD/PhD

Description: **This Project's purpose is to investigate the application of human embryonic and adult stem cells for vascular regeneration.**

Project Summary: General goal: To find clinically viable means creating arterial replacements optimal cell source, we are going to evaluate two types of stem cells: human bone marrow-derived mesenchymal stem cells (hMSCs) and human embryonic stem cell (hESCs)-derived biopotent mesenchymal stem cells. We have shown the feasibility of directing mesenchymal stem cells that are derived from adult human bone marrow down a vascular smooth muscle lineage. In addition, we have gone on to show the feasibility of using such cells to culture entire human arteries. However, the differentiation from MSC to SMC seems incomplete which lacks the expression of late contractile SMC markers, implying an intrinsic limitation of adult MSCs. The application of adult marrow-derived MSC in vascular regeneration is further hindered by their paucity in the marrow, unclear impacts of aging, and their limited passage number in vitro. Furthermore, the pathways that are involved in this differentiation process are not well understood. Human embryonic stem cell (hESC)-derived MSCs may be an attractive alternative due to their unlimited proliferative and differentiation capacity although it is not known whether mesenchymal stem cells that are derived from human embryos have the same vascular smooth muscle differentiation potential. In this application, we will utilize soluble factors, physical stimuli, and substrate matrix cues that are known to induce smooth muscle differentiation, and test their impact on the differentiation of mesenchymal stem cells derived from human embryonic stem cells. In addition, we will probe the signal transduction pathways of both adult and embryonic-derived cells, in order to determine their differences. Lastly, we will document the utility of vascular smooth muscle cells that are derived from human embryonic stem cells, for vascular tissue engineering. The results from these studies will not only inform the field whether hESC-derived MSCs share the same ability to undergo SMC differentiation as those from adults, but will also elucidate the signaling pathway responsible for SMC differentiation from mesenchymal stem cells (adult and embryonic). In addition, the implantation of the engineered vascular grafts in an immunodeficiency rodent model will represent the first in vivo assessment of human vessels engineered from embryonic and adult mesenchymal stem cells. The results could pave the way for development of a novel therapy for vascular disease.

O8-SCC-UCON-004

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Production and validation of patient-matched pluripotent cells for improved cutaneous repair.**

Amount requested: $1,960,890; Amount funded: $634,880
Principal Investigator Theodore P. Rasmussen, Ph.D.
Project P.I.s**:** Theodore Rasmussen, Ph.D., Winfried Krueger, Ph.D., Stephen Clark, Ph.D. Charles Giardina, Ph.D.
Institution: University of Connecticut (Storrs and Health Center)

Description: **This Project's purpose is to produce immunologically-matched pluripotent cells for the treatment of dermal lesions and other human cellular disorders.**

Project Summary: Regenerative medicine rests upon the ability to transplant immunologically matched cells into prospective patients. For this reason, substantial interest and effort has been focused upon the nuclear reprogramming of somatic cells, a process that can revert a patient's own cells to a state resembling an earlier developmental stage. Such reprogrammed cells exhibit two important properties: (1) they are pluripotent, meaning that they can be differentiated in vitro into a variety of therapeutically useful cell-types. (2) Since they contain the patient's own genome, they are immunologically matched to the patient and hence constitute a source of autologous transplantable cells. Several research avenues have been explored as means to produce patient-matched pluripotent cells. These include somatic cell nuclear transfer, fusion of somatic cells with ES cells, and the direct induction of pluripotency in somatic cells through the introduction of transgenes. This last approach, pioneered by Yamanaka and others, has led to the production of induced pluripotent stem-like (iPS) cells using a mouse system. Though these results are striking, they have not yet been adapted to a human system, and the use of oncogenic transgenes such as c-myc raises concerns about the safety of such cells. This grant application presents a plan to produce directly reprogrammed human cells (hDRCs) from human fibroblasts using novel approaches. We present strategies designed to identify human reprogramming factors that can be used for the induction of pluripotency in human fibroblasts without the use of oncogenes. We will assess the pluripotency and safety of these cells, and gain insights into the epigenetic nature of the reprogramming process over the course of this research. In addition, we propose to utilize hDRCs to begin translational research that involves the directed differentiation hDRCs and hESCs into cutaneous lineages. The production and use of such cells constitutes a tractable bioengineering goal, and will lead to the repair of human skin disorders such as burns, lesions, and other dermal conditions using autologous cells.

O8-SCC-YSME-005

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Translational Studies in Monkeys of Human Embryonic Stem Cells for Treatment of Parkinson's Disease**

Amount requested: $1,999,514; Amount funded: $1,120,000

Principal Investigator: **D. Eugene Redmond, Jr., M.D.**
Co-PI's: Robert H. Roth, Ph.D., John D. Elsworth, Ph.D., Csaba Leranth, M.D., Ph.D., Haifan Lin, Ph.D., Michael Snyder, Ph.D., Alfred Bothwell, Ph.D., Investigators: Jung H. Kim, M.D., Robert Makuch, Ph.D., Eleni Markakis, Ph.D., Caihong Qui, Ph.D.
Institution:**Yale University School of Medicine**
Collaborators: Steven Goldman, M.D., Ph.D., University of Rochester; Evan Y. Snyder, M.D., Ph.D., The Burnham Institute; Yang Teng, M.D., Ph.D., Harvard Med., Dennis Spencer, M.D., Ken Vives, M.D., Yale

Description: **This Group Project involving a collaboration among a group of senior Yale University investigators and Axion Research Foundation aims to develop a safe new human stem cell line that will effectively reverse a dopamine deficiency model of Parkinson's disease in monkeys, without side effects, toxicity, inappropriate migration, or immune rejection as a step toward translating this therapy to clinical treatment for Parkinson's disease.**

Project Summary: Our studies of human neural stem cells, which we have carried out since 1999 funded by USPHS Grant RO1NS040822, have been very promising, including finding the conversion of neural stem cells *in vivo* to cells with biological markers of dopamine neurons, preservation of endangered dopaminergic neurons and their striatal projections, production of key neuroprotective molecules, as well as selective migration and apparent normalization of abnormalities in cell sizes and numbers in the monkey's own nigrostriatal system. Some combination of these mechanisms also was associated with significant improvement in severely parkinsonian monkeys (PNAS, 104:12175-80, 2007). These findings suggest that functionally significant endogenous signals are present in the dopamine-depleted parkinsonian monkey brain to direct and sustain stem cell differentiation in ways that could be therapeutic for patients with Parkinson's disease. In order for human stem cells to move to clinical trials, a number of advances need to be made, all of which are possible with now published methods, and which we propose to carry out. Because all federally approved and fundable lines were derived and maintained with animal cells and products, they are considered potentially biohazardous and may not meet the standards of the National Academy of Sciences and the FDA. A new (Federally "unapproved") human stem cell line, developed and maintained under clinically-appropriate conditions, therefore, will be studied *in vitro* and *in vivo* in primates for full characterization, efficacy, and safety. We will determine an optimal level of differentiation that maintains or increases the functional success of our prior studies. We will study these cells, differentiated to make dopamine neurons *in vitro*, and compare two stages of differentiation in vivo in the best model of Parkinson's disease in monkeys. The cells will be characterized *in vitro* at several stages of development and differentiation, based upon state of the art biochemistry, pharmacology, histology, immunology, and genomics as a part of a Group Project. *In vivo* efficacy and safety studies in monkeys will be carried out by the PIs at the Axion Research Foundation's primate facility, using methods that were developed there and

extensively validated and published by our group. This project has the potential, and the investigators have the experience, to move to clinical trials in Parkinson's disease, if the experiments are successful, by the end of the proposed funding period.

O8-SCD-UCHC-003

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Flow Cytometry Core for the study of human Embryonic Stem Cells**

Amount requested $999,729; Amount funded: $250,000

Principal Investigator: Hector Leonardo Aguilia, Ph.D.
Institution: University of Connecticut Health Center

Description: **This Project's purpose is to organize a Flow Cytometry Core to provide services and collaborate in the identification, characterization and isolation of hESC and their derivatives.**

Project Summary: Flow cytometry is a powerful technique with the ability to identify rare cellular entities within complex populations of cells, to isolate them to homogeneity and to evaluate parameters as cell division, cell death and metablic functions.

The University of Connecticut Health Center established a Flow Cytometry Facility about 20 years ago to assist immunologists. At the present time, the facility is directed by Dr. Hector L. Aguila and Dr. Leo Lafrancois and provides services to scientists with research interests spanning most of the disciplines represented in the institution plus researchers from other institutions in the State. An increasing number of scientists with interests in Stem Cell Biology are active users of the facility, creating the need for analysis and sorting of hESCs and their derivatives. One of the hurdles to expand the services of the facility to hESCs is that the existing instruments are not optimal for handling live human cells, due to the lack of adequate aerosol containment systems. In addition, the growth of the facility has been supported by federal funds through direct purchasing of equipment or through the fees charged to users. This limits the use of instruments to fixed samples and for many instruments to exclusively federally approved hESC lines. The institution has been responsive to the needs and the interest in hESC research and has recently invested in the purchasing of an Aria cell sorter instrument I(Becton Dickinson, San Jose, Ca). This state of the art instrument is custom designed to perform applications especially suited for hESC research and it will be dedicated mostly to the analysis and sorting of hESCs and their derivatives.

This proposal will seek support for establishing a core to provide advice, training and services on flow cytometry to stem cell researchers. Beyond services, priority will be given to establish active collaborations with multiple investigators to develop novel flow cytometry applications for studying properties of hESCs and their derivatives. These include: profiling and selection of undifferentiated hESCs, new detection techniques to evaluate expression of endogenous fluorochromes (i.e. green fluorescent proteins expressed in the context of developmentally regulated promoters) and cell surface markers with antibodies coupled to multiple fluorochromes. This core will interact closely with the institutional hESC Core in screening existing and newly generated cell lines to design quality control parameters and enhancing the educational mission of the core.

O8-SCD-YALE-004

CT Stem Cell Research Proposal
Project Summary (in Non-Scientific Language)

Title of Project: **Maintaining and Enhancing the Human Embryonic Stem Cell Core at the Yale Stem Cell Center**

Amount requested: $2,500,000; Amount funded: $1,800,000

Principal Investigator: **Haifan Lin, Ph.D.**
Institution **Yale University**
Collaborator: Diane Krause, M.D., Ph.D.

Description: **This proposal requests continued support of the human embryonic stem cell core facilities for their continued operation through May 2011 and to improve one of them (the Genomics Core) to meet the current demand of stem cell research in Connecticut.**

Project Summary: This application requests the continued support of the following four established core laboratories beyond the current funding periods to allow their continued operation to May 2011: (1) The hESC Core, already up and running, is eliminating the overfull situation of the UConn hESC core in cell line distribution and training. In addition, it will develop new hESC lines, new protocols, and transgenic/gene knock-out technologies to meet the pressing needs of stem cell research in the State. (2) The Confocal Core, which is the only non-federally funded cell imaging facility in the State dedicated to hESC study. (3) The FACS Core, representing over $0.8 million of support from Yale to compensate for the budget reduction of the 2006 Core Grant to Yale, is the only cell sorting/analyzing system at Yale, and perhaps in the State, that is purchased with non-federal funds and can be used for hESC research on non-registered hESC lines. (4) The Genomics Core, supported by a 2006 Hybrid Grant, is the only genomic core for hESC research in the State. The continued operation of these Cores is essential for hESC research in the State. In addition, this application requests funds to enhance the Genomics Core by adding a Solexa deep sequencing machine (~ $480,000) to meet the pressing needs of over 50 stem cell labs in the State for this new technology. The addition of this instrument will propel both academic and industrial stem cell researchers in the State to the forefront of the genomic and genetic research of stem cells. In addition, it will allow the Connecticut Stem Cell Initiative to dovetail with the Connecticut Genomics Initiative.